# The giant SALR cluster fluid: A review

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We review recent developments in understanding the behaviour of equilibrium fluids with competing short-range attractions and long-range repulsions at low concentration. Typically, this SALR model system is used to represent solutions where at least one of the solute species interacts via a screened-coulomb potential, which includes many colloidal, nanoparticle and polyelectrolyte solutions among others. Provided neither the short-range or long-range interactions are too strong, and the concentration remains sufficiently low, then discrete spherical clusters of solute are expected, here called "Giant SALR clusters". Because these clusters can themselves be disordered, as though fluid particles with a renormalised interaction, such cluster fluids cannot be treated using standard mean field methods. Our review therefore focuses on theoretical routes that aim to overcome this difficulty. We highlight the main outcomes of these theories and discuss how they relate to soft matter and especially biology, including complex coacervates, membraneless organelles and the origin of life.

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## I. INTRODUCTION

Many disparate physical systems consist of mobile particles interacting through attractive and repulsive forces that compete over different length-scales. In each case, provided neither the attractions or repulsions are too strong, giant clusters can be observed under suitable conditions. The most obvious example is atomic nucleii. Here, the strong nuclear force causes protons and neutrons to aggregate, but coulomb repulsion between the protons limits the size of aggregates that form to those atomic nucleii that appear in the periodic table of elements. Moving up in length-scale, we find similar clustering behaviour in many kinds of solution, including biological and soft matter, where the solutes now include charged colloids, nanoparticles, macromolecules, proteins, and their mixtures[1,2]. At the macroscale we find similar behaviour in intelligent agents, including mobile autonomous robots, birds and even humans[3,4], that form swarms or communities. Clearly, this kind of pattern formation is ubiquitous and important. Recent work suggests it might even be relevant to the origin of life.

If either the interparticle attractions or repulsions are too strong, relative to the motive force, then gels, glasses and other kinds of kinetically frustrated aggregation patterns emerge. They have been studied extensively, especially for soft matter systems. Conversely, if both the attractions and repulsions are too weak, then, obviously, we observe only uniform fluid dispersions. Between these limits, if particles remain sufficiently mobile that they can quickly and easily make and break weak bonds with neighbouring particles, we can find interesting equilibrium phase behaviour. Our specific interest here is on those equilibrium systems where short-range attractions and long-range repulsions roughly balance to create structures with a length-scale much larger than the particles involved. The phases created remain bulk phases, its just that pair correlations occur on scales much longer than the individual particles. At low concentrations, structure typically appears in the form of a disordered dispersion of discrete "blobs". We call this phase the "cluster fluid", and it is this phase that we are most concerned with here. These clusters are distinguished from the small clusters of, typically, just a few particles that occur transiently in any fluid by a free energy barrier. That is, the cluster size distribution has a minimum between one particle and the equilibrium size of giant SALR clusters. At equilibrium, such that discrete giant clusters are able to exchange particles on a relevant timescale, the clusters will be statistically identical in the absence of external forces. Fortunately, atomic nucleii equilibrate with each other very slowly, and we will therefore not discuss them any further.

By considering only cluster fluid phases at equilibrium, we are typically limited to soft matter and biological systems consisting of colloids, nanoparticles and biological macromolecules. But it is likely this behaviour extends also to other solutions not conventionally included in this realm, such as aqueous solutions of relatively small ions and molecules. Cluster fluid phases, if they form, can be much harder to unambiguously identify for these small-molecule systems because the clusters formed

can be smaller than the resolution threshold of even the best optical microscopes. Nevertheless, there is no reason in principle why the cluster fluid phase should not be prevalent in these solutions, and it is conceivable that many examples of the cluster fluid phase have been overlooked in small-molecule solutions.

The first statistical thermodynamic theory capable of describing the giant SALR cluster fluid phase was published in 2014. Prior to this development, theories were typically either phenomenological or focused on the formation of individual clusters rather than cluster fluid phase behaviour. Moreover, giant SALR clusters are unfamiliar territory for most scientists, and their behaviour and potential for widespread applications remain largely unexplored. Consequently, we begin our review of recent developments, which focuses on work since 2014, with a review of the state-of-play up to this point.

## II. HISTORICAL BACKGROUND

One of the first systems demonstrated to form finite sized clusters at equilibrium is the aqueous surfactant solution[5]. When increasing the concentration of surfactant molecules (which are surface active) in water, abrupt, qualitative changes occur in various properties of the solution when the concentration increases above a threshold value, known as the critical micelle concentration (cmc). For example, the electrical conductivity of the solution will increase roughly linearly with surfactant concentration up to the cmc. Above the cmc, however, the slope of the conductivity drops suddenly to a lower value. A similar phenomenon (i.e. a break in the slope at the cmc) occurs with other properties, including the surface tension and the osmotic pressure of the solution.

McBain first suggested[6–8] that this change in the behavior of the solution was due to the formation of clusters by the surfactant molecules. The conductivity is related to the number of free charge carriers and their rate of diffusion (which, in turn, is related to their size), while the osmotic pressure is related to the effective number of free particles in the solution. Both of these quantities will 'stall', or hardly change, as surfactant concentration is increased above the cmc.

Surfactant molecules are typically composed of two different types of moieties: a hydrophilic portion, which likes to interact with water, and a hydrophobic portion, which water does not like to interact with. If these two parts of the surfactant were not chemically bonded, they would separate to form two immiscible phases, similar to oil and water. This tendency for the hydrophobic portion of the surfactant molecule to avoid unfavorable contact with water will lead to the formation of a cluster of surfactant molecules, where the hydrophobic groups are in the interior and are shielded from contact with water by the hydrophillic portions of the molecules. This is known as a micellar aggregate, or a micelle, and is favored energetically.

While the formation of a micellar aggregate is favored energetically, the confinement of the surfac-

tant molecules within the cluster entails an entropy penalty. This penalty increases as the number of molecules within the cluster increases. At low surfactant concentrations, the entropy cost dominates the solution, as only a few molecules with a lot of accessible space need to be confined in a relatively small region. In this case, the energy benefit is insufficient to promote the formation of clusters. At high surfactant concentrations, the cost of confinement is not so great, as the environment is already relatively crowded. Consequently, the energy benefit will dominate, and cluster formation will be favorable. This balance between energy and entropy leads to the critical micelle concentration, the surfactant concentration above which clusters will form to a significant degree in solution. The sharpness of the transition increases with the size of the aggregates. The formation of clusters does not denote a discontinuous transition, due to the fact that the clusters have a finite size.

An early theoretical model for describing the formation of micellar aggregates was developed by Debye[9–11]. In this approach, the aggregation of surfactants to form a micelle is treated as a chemical reaction, where each micelle is considered to be a distinct chemical species with its own free energy of formation. To illustrate the theory, we consider a surfactant solution which forms micelles of a single size, consisting of $n$ surfactant molecules. In this case, the Helmholtz free energy of the system can be written as

$$F = N_1\mu_1^{\ominus} + N_n\mu_n^{\ominus} + F_{\mathrm{mix}}$$

where $N_1$ is the number of monomers, $\mu_1^{\ominus}$ is their formation free energy, $N_n$ is the number of clusters, $\mu_n^{\ominus}$ is their formation free energy, and $F_{\mathrm{mix}}$ is the free energy change of mixing the particles and clusters together.

Minimization of the free energy with respect to the micelle concentration, while maintaining the constraint that the total surfactant concentration must remain constant:

$$c_s = c_1 + nc_n.$$

leads to the reaction equilibrium criterion:

$$\mu_n = n\mu_1. \tag{1}$$

This is identical to the relation that would be found for chemical reaction equilibrium, which reinforces the idea that the cluster is a separate species that forms from individual particles.

If we neglect the interaction between particles and cluster (assuming that the system is ideal), the chemical potentials of the monomers and the micelles are

$$\beta\mu_1 = \beta\mu_1^{\ominus} + \ln\frac{c_1}{c^{\ominus}}$$
$$\beta\mu_n = \beta\mu_n^{\ominus} + \ln\frac{c_n}{c^{\ominus}}.$$

where $\beta = 1/(k_B T)$, $k_B$ is the Boltzmann constant, and $T$ is the absolute temperature of the system. Then, Eq. (1) can be written as

$$\frac{c_n}{c^{\ominus}} = \left[\frac{c_1}{c^{\ominus}}\right]^n e^{-\beta n g_n}$$

where $c_1$ is the concentration of monomers, $c_n$ is the concentration of clusters, $c^{\ominus}$ is a reference concentration (e.g., 1 M), and $g_n = \mu_n^{\ominus}/n - \mu_1^{\ominus}$ is the free energy of micellization (the free energy cost to form an isolated micellar aggregate from isolated monomers). Substituting this expression into the surfactant balance expression, we find

$$\frac{c_s}{c^{\ominus}} = \frac{c_1}{c^{\ominus}} + n\left(\frac{c_1}{c^*}\right)^n.$$

In this case, we find we can identify $c^* = c^{\ominus} e^{\beta g_n}$ with the critical micelle concentration. At surfactant concentrations below the cmc, the second term on the right is much smaller than the first, and so the monomer (unaggregated) surfactant concentration $c_1$ is nearly equal to the total surfactant concentration $c_s$. Above the cmc, the second term becomes much larger than the first term, and most of the surfactants will be within micellar aggregates. The monomer concentration, however, will be slightly higher than the cmc. As the aggregation number $n$ of the micellar aggregate becomes larger, the transition of the solution properties near the cmc will become sharper and more clearly defined.

This approach can be easily extended to describe the possibility of forming a range of aggregates, by introducing concentrations $c_{n'}$ and formation free energy $g_{n'}$ terms for other sized and shaped aggregates, and was in fact done by Debye in order to estimate the degree polydispersity. Key to the theory are the expressions for the free energies of formation $g_n$ of the aggregates. In the original theory developed by Debye, he estimated the free energy of formation based on a lamellar structure for the micellar aggregate proposed by McBain, and put forward simple estimates of the work needed to overcome the charge of the hydrophillic "head" groups and the energy gained back through the contact between the hydrophobic "tail" groups.

In early applications of this theory of micellar solutions, the formation free energies were based on more macroscopic properties, such as the free energy of solvation of hydrocarbons or the water-hydrocarbon interfacial tension[12,13] Later, molecular statistical mechanical approaches were used to develop expressions for the free energy of formation of micellar aggregates of different sizes[14]. In these systems, the interactions between clusters are typically not important, and the solution is assumed to behave ideally. However, later works have looked to include the interactions between the micellar aggregates in the theory[15]. An excellent review of the extensions and advances in the theory of micellar aggregates is given by Nagarajan[5]. This includes the formation of worm-like micelles, surfactants of different architectures (e.g., bolaamphiphiles, gemini surfactants, etc.)

So far, our discussion of various descriptions of cluster forming systems have been limited exclusively to surfactant solutions or solutions composed of molecules with two chemically incompatible

groups that are bonded together within a molecule (e.g., block co-polymers). In these systems, the formation of the clusters is mainly driven by the net "attraction" between one section of the molecules (e.g., the tail groups). For the surfactant and block co-polymer systems, the driving force of the formation of clusters is the effective "attraction" between the tail groups of the amphiphiles. The effective repulsion between the head groups dictates the geometry of the resulting cluster. However, attractive interactions are not necessary for cluster formation.

Particles interacting through core softened potentials, where there are only repulsive interactions and no attractive interactions, have been shown to also exhibit cluster formation. Colloidal particles with polymer chains in good solvent conditions grafted to their surfaces interact through these types of potentials. Cluster formation in these systems are driven entirely through entropy. The simplest example of a core softened potential is the square-shoulder potential, where a hard sphere system has an additional repulsive, step interaction of finite width. This purely repulsive interaction potential exhibits a rich range of ordered structures, which have been examined using molecular simulation and density functional theory[16,17]. Minimum energy configurations have been examined using genetic algorithms for two-dimensional[18] and three-dimensional[19] systems.

Clusters can also form in single hydrophobic polyelectrolytes, which are polymers formed of hydrophobic monomer groups with charged groups (either all positive or all negative) interspersed at various places within the chain. In this case, the hydrophobic groups will tend to collapse the chain, while the similarly charged monomers will tend to separate the monomers. This leads to the formation of "beads" along the chain, where each bead is a section of polymer collapsed into a spherical cluster separated by linear connecting stretches, as observed through molecular simulations[20–24]. The size of these beads is controlled by the linear charge density and the strength of the attractive hydrophobic interactions[25–28].

Systems that form clusters can also be "designed". That is, the particular interaction potential between particles that is required to yield a target, equilibrium cluster structure can be determined. Using an inverse Boltzmann strategy for target particle correlation functions, Jadrich and coworkers were able to find an interaction potential that corresponds to monodisperse aggregates[29]. These potentials were found to have a fairly broad attractive well with a relatively sharply peaked repulsive interaction. A similar strategy can be used to design "inverse" materials (i.e. porous materials with well-defined voids[30].

Finally, we note that systems of particles that interact with each other through a potential with a short-range attraction and a long-range repulsion (SALR) can exhibit clustering, which is the main focus of this review. These type of interactions appear in a wide variety of physical systems. An important example is the interaction between charged colloidal particles in aqueous salt solutions, which is typically given by the Derjaguin-Landau-Verwey-Overbeek (DLVO)[31,32] theory. In this case,

the short-range attraction arises from dispersion (van der Waals) forces. The long-range repulsion comes from the Coulomb interaction of the charges, which are screened by the presence of the salt; the Debye screening length sets the range of the interaction. DLVO theory has also been used to describe the behavior of many other systems, including proteins, micellar aggregates formed from ionic surfactants, nanoparticles, cells, etc.

Computer simulation studies indicate that SALR potential systems can form clustered structures at equilibrium[2,33,34]. The minimum energy configuration of clusters of different number of particles[2,33] give an indication of the propensity of the system to form cluster phases. In the absence of the repulsive interaction, the energy of the clusters decreases monotically as the size of the clusters increase. However, with the addition of a long-ranged repulsion, these cluster energy curves can exhibit a minimum at a particular size, indicating that the system has a propensity to form clusters of that size. Analysis of the variation of pressure with the particle density from simulation studies reveals that there are two types of clustering behaviour[34]. The first is in line with what is seen in surfactant systems, where the pressure has a distinct break with particle density when clustering begins to occur. This corresponds to the case where short-range attractions dominate, the clusters do not interact strongly with each other, and the pressure essentially is a measure of the number of clusters. In the other type of behaviour, where long-range repulsions dominate and primarily drive the formation of aggregates, there is no break in the pressure associated with the onset of clustering.

SALR potential systems are not only able to form disordered equilibrium cluster phases but also can exhibit a wide variety of ordered mesophases (e.g., lamellar, fcc cluster phase, etc.) similar to the lyotropic liquid crystalling phases formed by aqueous surfactant solutions discussed previously[35].

Many different mathematical forms can be used to represent an SALR potential. One of the first SALR potentials that was used to examine the possibity of clustering was a sum of a Lennard-Jones $2\alpha$-$\alpha$ potential, with $\alpha$ ranging from the typical value of $6$ up to values on the order of $100$, and a repulsive Yukawa tail[36]. The Lennard-Jones potential provides the excluded volume interactions, which prevents the particles from overlapping, and a strong, short-ranged attractive interaction, while the Yukawa potential gives the long-ranged repulsion. Another SALR potential that has been examined is a hard sphere with a narrow square well attraction with a triangular (constant force) repulsive potential[35].

The particular SALR potential that has received the most attention has been systems of hard spheres with a diameter $d$ that interact with each other through a potential composed of two Yukawa potentials[37–40]. For two particles whose centres are separated by a distance $r$, the interaction energy $\phi(r)$ is given by:

$$\beta\phi(r) = A_r \frac{e^{-\kappa_r(r-d)}}{r/d} - A_a \frac{e^{-\kappa_a(r-d)}}{r/d} \tag{2}$$

where $A_a$ is the magnitude of the attractive interaction, $\kappa_a^{-1}$ is the range of the attractive interaction,

$A_r$ is the magnitude of the repulsive interaction, and $\kappa_r^{-1}$ is the range of the repulsive interaction.

In the next section, we will discuss a statistical thermodynamic approach for treating the phase behaviour of the giant SALR cluster fluid phase. Our focus is on a recent "multi-scale" approach developed by Sweatman and co-workers[41,42] which treats the cluster fluid as a mixture of primary particles and giant clusters, which are themselves formed of the primary particles. To our knowledge, it is the first theory capable of describing the phase behaviour of the giant SALR cluster fluid in terms of interactions between the primary particles alone.

## III. THEORY

The basic framework of the theory is similar to that of the initial statistical thermodynamic description of micellar aggregates. The main difference being the important role that interactions between the clusters, and between primary particles and clusters, plays in both forming SALR clusters and influencing cluster phase behaviour. While this approach is fairly successful, it does have limitations, however, and we end the section by discussing these and other directions in developing theories for clustering systems.

### A. General framework

Physically, the clusters are considered as a separate chemical species, with their own free energy of formation and interaction potential with other chemical species (e.g., single SALR particles or other clusters). To develop a free energy model of the system, we conceptually separate this into a contribution due to the creation (self energy) of the species and a contribution due to the mixing and interaction of species in the system:

$$F = N_v \mu_v^{\ominus} + \sum_{\alpha} N_\alpha \mu_\alpha^{\ominus} + F_{\text{mix}} \tag{3}$$

where $N_v$ is the number of monomer particles (i.e. particles that do not belong to a cluster) in the system, $\mu_v^{\ominus}$ is the formation free energy of a single, isolated particle, the index $\alpha$ runs over the types of clusters in the system, $N_\alpha$ is the number of clusters of type $\alpha$, $\mu_\alpha^{\ominus}$ is the formation free energy of a cluster of type $\alpha$, and $F_{\text{mix}}$ is the free energy of mixing the particles together. The formation free energy of a single SALR particle $\mu_v^{\ominus}$ is taken as the reference state. In order to simplify the description of the theory, we limit ourselves to the situation where only one type of cluster is formed.

## B. Clusters

We begin by estimating the free energy of formation for a cluster of SALR particles. This requires that we specify the structure of the cluster, fixing its size, geometry, etc. This is given by the distribution function $P(\mathbf{r})$, which gives the probability density of a particle in a cluster centered at the origin to be located at position $\mathbf{r}$. For example, let us assume that the clusters are spherical with a diameter $d_c$ and contain $n$ particles. If we assume that the cluster boundary is sharp, then the volume of the cluster is $V_c = \pi d_c^3/6$, the density of particles within the cluster is $\rho_l = n/V_c$, and $P(\mathbf{r}) = \rho_l \Theta(d_c/2 - |\mathbf{r}|)$, where $\Theta(r)$ is the Heaviside step function. Typically, particles within a cluster will be fairly dense; for simple liquids in this regime, the structure is dominated by excluded volume interactions, so we expect that the pair correlation function should be well approximated by that of a hard sphere fluid.

The formation free energy (or self energy) of each cluster can be formally divided into an energetic contribution and an entropic one,

$$\mu_c^{\ominus} = U_{\text{self}} - TS_{\text{self}}$$

while the interaction energy of particles within a cluster is then given by

$$U_{\text{self}} = \frac{1}{2} \int d\mathbf{r} d\mathbf{r}' P_c(\mathbf{r}) \phi(\mathbf{r} - \mathbf{r}') g_{\text{HS}}(\mathbf{r} - \mathbf{r}') P_c(\mathbf{r}')$$

The entropy of the cluster is approximated as

$$S_{\text{self}} = S_{\text{cm}} + n s_{HS}(\rho_l, d)$$

where $V_c$ is the volume of the cluster, $s_{HS}(\rho, d)$ is the specific configurational contribution to the entropy of a uniform fluid of hard spheres of diameter $d$ at number density $\rho$ (the momentum contribution to the entropy is already included in the reference state), and $S_{\text{cm}}$ is the entropy cost of fixing the centre of mass of the particles of the cluster.

## C. Mixing of monomers and clusters

The free energy of mixing is also divided into energetic and entropic contributions:

$$F_{\text{mix}} = U_{\text{mix}} - TS_{\text{mix}}.$$

The internal energy of mixing $U_{\text{mix}}$ is due to the interaction between particles; it excludes the interactions between particles within the same cluster, which is already accounted for in the cluster formation free energy. The effective interaction energy between a monomer SALR particle and a cluster is given by:

$$u_{vc}(|\mathbf{r} - \mathbf{R}'|) = \int d\mathbf{r}' \phi(|\mathbf{r} - \mathbf{r}'|) P(\mathbf{r}' - \mathbf{R}'), \tag{4}$$

while the effective interaction energy between clusters is

$$u_{cc}(|\mathbf{R}-\mathbf{R}'|) = \int d\mathbf{r}d\mathbf{r}' P(\mathbf{r}-\mathbf{R})\phi(|\mathbf{r}-\mathbf{r}'|)P(\mathbf{r}'-\mathbf{R}'). \tag{5}$$

The internal energy of mixing is then given by

$$\begin{aligned} U_{\text{mix}} = \frac{\rho_v^2}{2}\int d\mathbf{r}d\mathbf{r}'\phi(|\mathbf{r}-\mathbf{r}'|)g_{vv}(|\mathbf{r}-\mathbf{r}'|) + \rho_v\rho_c\int d\mathbf{r}d\mathbf{r}' u_{vc}(|\mathbf{r}-\mathbf{r}'|)g_{vc}(|\mathbf{r}-\mathbf{r}'|) \\ + \frac{\rho_c^2}{2}\int d\mathbf{r}d\mathbf{r}' u_{cc}(|\mathbf{r}-\mathbf{r}'|)g_{cc}(|\mathbf{r}-\mathbf{r}'|). \end{aligned} \tag{6}$$

where $g_{vv}(r)$ is the pair correlation function between between monomer particles, $g_{vc}(r)$ is the pair correlation function between a monomer particle and a cluster, and $g_{cc}(r)$ is the pair correlation function between clusters. The pair correlation functions in the monomer-cluster mixture interacting with the SALR potential and the effective potentials given in Eqs. (4) and (5) can be approximated through various means, such as the EXP approximation or the optimised random phase approximation, based on a hard sphere mixture as a reference system[41,43,44].

The final term to consider is $S_{\text{mix}}$, which is the entropy change due to mixing the monomer SALR particles with the clusters. This can be estimated by considering separately the mixing entropy of the clusters and the mixing entropy of the monomer SALR particles in the volume left by the clusters. Both of these can be estimated based on the properties of hard sphere fluids:

$$S_{\text{mix}} \approx N_c s_{HS}(\rho_c; d_c^{\text{eff}}) + N_v s_{HS}(\rho_g; d) \tag{7}$$

where $s_{HS}(\rho, d)$ is the specific entropy of hard spheres of diameter $d$ at density $\rho$, $\rho_g$ is the effective density of the monomer SALR particles, and $d_c^{\text{eff}}$ is an effective hard sphere diameter that characterises the interactions between the clusters; it is given by the Barker-Henderson criterion[43]

$$d_c^{\text{eff}} = \int_0^\infty dr[1 - e^{-\beta u_{cc}(r)}].$$

This completes the specification of the free energy of mixing.

### D. Parameter optimisation

In the previous sections, we developed an approximation for the free energy of a clustering SALR system. However, the extent to which clustering occurs and the precise geometry of the clusters is still unknown. To determine these properties, we use the fact that the equilibrium state of a closed system held at fixed temperature and volume minimizes its Helmholtz free energy. In practical terms, this means that we minimize the free energy of the system with respect to the cluster parameters in the model, which are specifically the fraction of particles that are within clusters, the diameter $d_c$ of the clusters, and the number of particles $n$ in each cluster (or equivalently, the density $\rho_l$ of particles

within a cluster). This minimisation needs to be performed under the constraint that the total number density $\rho_b$ of particles in the system remains fixed, which implies

$$\rho_b = \rho_v + n\rho_c \tag{8}$$

where $\rho_v$ is the number density of monomer particles, and $\rho_c$ is the number density of clusters in the system.

Once these clustering parameters have been determined, the theory is complete. In section V, the predictions of the theory will be discussed in detail. However, we will discuss other possible directions in which to model clustering next.

### E. Other approaches

The theory we have presented is based on a molecular thermodynamic framework which considers clusters as separate "chemical species". Although the molecular thermodynamic approach, presented in this section specifically for SALR systems, has been quite successful in describing the structure and phase behaviour of systems that exhibit clustering, there are some conceptual and practical difficulties with the approach for more general systems. One of the difficulties is the need to define *a priori* what is meant by a cluster and to precisely specify its geometry. This does not pose a difficulty in cases where the clusters are fairly well defined and rigid. However, difficulties occur when the boundaries of the clusters are more diffuse or when the clusters are flexible, such as in the case of wormlike micelles. This creates ambiguity as to what particles belong to a cluster and what particles are monomers, making it difficult to formulate approximations for the formation free energy and the mixing free energy. It would be preferable to have a theory that would more "naturally" exhibit cluster formation, without the need to specify the characteristics of the aggregates in advance.

Another approach that has been quite successful in modelling the properties of simple liquids are integral equation approaches, such as the Percus-Yeckick and hypernetted chain approximations[43]. Typically, the Ornstein-Zernike equation is coupled with an approximate "closure" relationship, which is crucial to the theory. While these methods have progressed substantially, able to describe the properties of some complicated molecular systems, they still struggle for systems with where there are correlations with ranges substantially larger than the typical size (or interaction range) of the constituent particles. This is a key characteristic of a clustering system.

Integral equation approaches have been applied to examine SALR systems[45–48]. While they do show indications of clustering, they have not been able to quantitatively capture the structure and thermodynamics of the system when clustering becomes significant. The main difficulty with the integral equation approach is in physically motivating the various approximations used to develop the

closure relation. Typically, they are designed for mathematical convenience, or attempt to provide some physical constraints, such as thermodynamic consistency. Consequently, without physical insight to guide the improvement of approximate closure relations, integral equation methods appear to be a difficult route to pursue.

Another possible approach is to use density functional theory, which develops expressions for the free energy functional of non-uniform distributions of species. The equilibrium density profile then corresponds to the one that minimizes the free energy. These theories are capable of predicting a wide variety of phenomena[49], including capillarity and crystallisation. This approach has been employed to model the behaviour of aqueous surfactant solutions[50] and block co-polymer systems[51]. A recent review of the theory of block co-polymer micelles is given in Ref. 52. Interestingly, the connectivity of the head and tail groups within the surfactants due to chemical bonding is found to strongly resemble electrostatic interactions[53]. This connectivity term tends to homogenise or mix the system at large length scales, while the chemical incompatibility of the head and tail groups tends to segregate the system at short length scales. This competition leads to the formation of clusters.

Density functional theories can predict the formation of cluster phases. At low concentrations, the uniform density profile minimizes the free energy functional. At higher concentrations, non-uniform density profiles can minimise the free energy functional. From more general, field theoretic considerations[1], the key element for the formation of "modulated phases", where there is segregation at some mesoscopic length scale, has been found to be the presence of competing interactions (e.g., attractions and repulsions) with differing length scales, which occurs in a wide variety of physical systems. This competition may lead to a new length scale at which segregation (e.g., clustering) will occur. This perspective has been applied directly to particle systems in order to elucidate the kind of interaction potentials that can lead to the formation of mesophases, which are segregated at intermediate length scales[54]. In the case of surfactant solutions or systems of SALR particles, these can correspond to the formation of mesophases composed of regularly organised arrangements of clusters, such as lamellar, hexagonal, or cubic lyotropic, liquid crystalline phases formed by aqueous surfactant solutions or block copolymer melts and blends. Note that these are, however, translationally invariant structures and not the isotropic cluster phases that have been considered in this section.

The key conceptual difficulty in applying density functional theory directly to describe cluster formation in isotropic solutions, such as in aqueous micellar solutions, is that for isotropic systems the free energy functional should always be minimised by a uniform density profile. The presence of non-uniform density profiles that lower the free energy functional implies that the isotropic solution is no longer thermodynamically stable.

While density functional theory, as currently formulated, is unable to directly model isotropic cluster systems, it is able to describe the nucleation of clusters, such as micelles in aqueous surfactant

solutions[55–58] and in SALR systems[59]. This theory can describe not only spherical micellar aggregates, but also more complicated structures, such as vesicles, as well as ordered mesophases lamellar phases. Density functional theory has also been used to predict the formation of block copolymer[60] micelles. These and related applications of density functional theory, however, have only been used to predict the free energy of formation of individual micelles, and, consequently, fits into the general thermodynamic framework described in this section. More work still needs to be done to directly apply density functional theory to isotropic cluster phases, although some interesting approaches to this problem have been proposed[61–63].

## IV. SIMULATION METHODS

Any molecular simulation method, such as molecular dynamics, Brownian dynamics or Monte Carlo (MC) simulation, can be used to simulate the cluster fluid phase of SALR fluids. However, equilibration can be slow, especially for fluids with larger clusters. These systems present two particular issues.

First, the larger the cluster the more highly coordinated its motion becomes. Therefore, when using standard Monte Carlo simulations which involve only single-particle moves, equilibration of cluster degrees of freedom can become very slow. Fortunately, there are several effective methods for dealing with this problem. A particularly straightforward approach involves the design of "cluster moves", where an entire cluster is translated and/or rotated within a single MC move. Many schemes have been devised for performing cluster moves that satisfy detailed balance, including the so-called "force-bias" cluster move[64]. Our work, however, uses a particularly simple scheme as follows[41]. First, a particle is chosen at random. Then, we attempt to translate all particles within a particular distance of this particle by the same vector, chosen randomly from within a sphere of pre-defined radius. The move is accepted according to the usual Boltzmann criteria but is automatically rejected if the list of particles for the reverse move is different (which would occur of the cluster is moved too close to an isolated particle).

A second issue is equilibration of particles between clusters. As cluster size increases, individual particles in the background fluid experience an increasingly repulsive interaction with clusters. Essentially, a corona of reduced particle density surrounds each cluster that particles rarely cross. Likewise, particles at the surface of a cluster become increasingly trapped as cluster size increases. This issue affects all standard dynamical simulation methods but can be partially ameliorated when using Monte Carlo simulation[41]. In this case, a fraction of particle translation moves can be chosen to be particularly large. Particles can then effectively 'hop over this barrier. These MC moves also have the benefit of equilibrating particles in the background vapour phase more efficiently. Since small

translation moves are required to equilibrate particles within clusters where the concentration of particles can be much higher than in the background fluid, an efficient strategy is to randomly select, according to a fixed probability, between large and small translation moves.

Consequently, Monte Carlo simulations are typically preferred for equilibration of cluster phases. Of course, dynamical simulations should be used instead if dynamical properties are of interest. For soft matter systems involving colloids and nanoparticles dispersed within a solvent, Brownian dynamics is a natural choice if explicit solvent degrees of freedom are unimportant. However, it is now understood that Monte Carlo simulation provides a very good simulacrum of Brownian dynamics provided only small translational MC moves are attempted (i.e. the special MC moves described above are not used). In this case, procedures have been developed to link the MC step size to a timescale for fluids with spherical particles[65,66]. For this reason, and given the versatility of Monte Carlo simulation for treating particles with hard cores or other discontinuous inter-particle interactions, we have used Monte Carlo simulation in all our work in preference to other methods. When dynamical properties are of interest we simply switch off any special MC moves, thus mimicking Brownian dynamics (at least for spherical particles).

## V. PHASE BEHAVIOUR

We are concerned here with the behaviour of SALR fluids at low concentration where distinct spherical clusters form an equilibrium cluster fluid. Behaviour at higher concentrations is relatively well understood, and appears to be quite similar to surfactant systems, i.e. wormlike and lamellar structures can form at equilibrium. These modulated structures persist up to high concentrations. However, before the uniform liquid phase is obtained at the highest concentrations, we expect to find the "inverse" cluster fluid[30], i.e. an isotropic fluid of spherical voids embedded within the surrounding liquid. We expect this situation can also be studied with the methods described in the preceding section.

The first molecular thermodynamic theory able to describe the cluster fluid phase of SALR fluids was published in 2014[41]. We therefore focus on the results of this work, and later work that builds on it, in the following subsections. All this work employs the 2-Yukawa SALR pair potential described in section II, and therefore all units are in reduced form relative to the hard sphere diameter $d$ and thermal energy scale $k_BT$. As described in section III, theories of micellization typically focus on the formation free energy of a single cluster, and so do not address the question of cluster fluid phase behaviour. That is, they do not take account of interactions between clusters, or between the remaining monomers and clusters, and the free energy of their mixing. Likewise, some useful insight into low-concentration phase behaviour is provided by integral equation methods[47]. However, current recipes

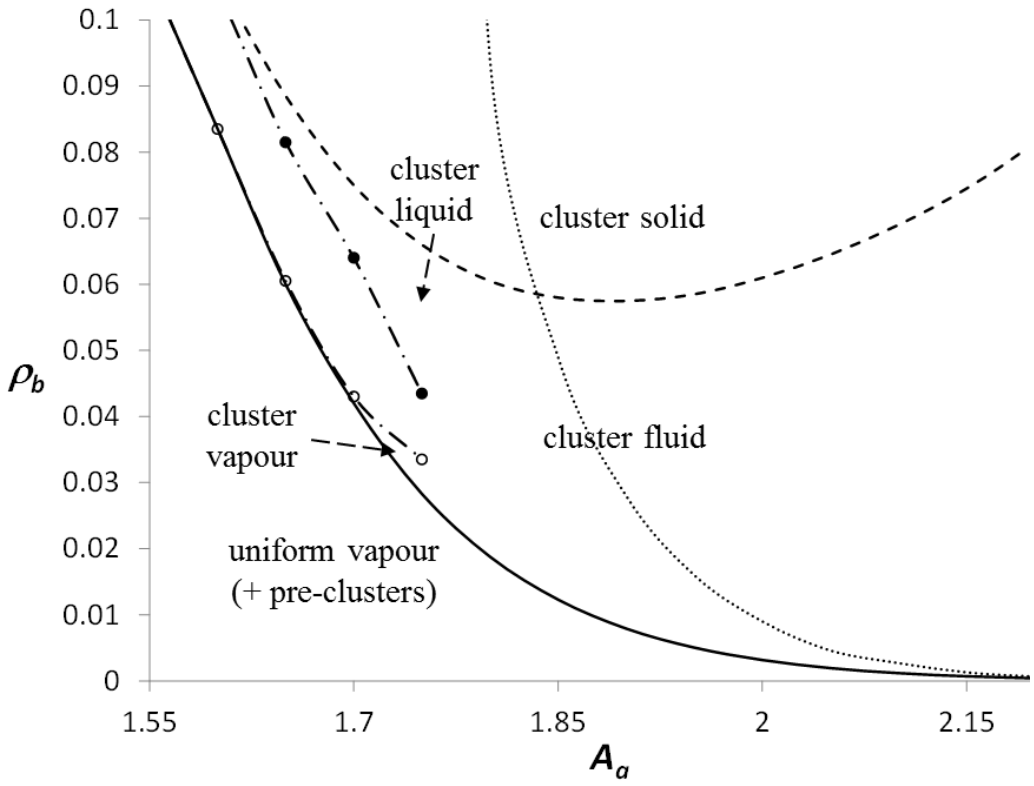


Figure 1. Generic phase diagram for single-component SALR fluids at low concentration. The overall system density, $\rho_b$ is on the vertical axis while $A_a$ on the horizontal axis denotes the strength of short-range attractions. The three main regions are the uniform vapour (with pre-clusters), cluster fluid and cluster solid. A first-order cluster vapour to cluster liquid transition is also predicted. The dotted line describes the binodal for a bulk vapour-liquid phase transition.

for the bridge function appear unsuitable for the cluster fluid phase more generally.

### A. Single component

Figure 1 shows the phase diagram resulting from the theory described in section III. Due to the large number of model parameters only a restricted view of the SALR cluster fluid phase diagram is possible. Here we show the competing effects of overall system concentration, or bulk density $\rho_b$, and the strength of attractive interactions, $A_a$.

The main feature of the phase diagram is the critical cluster concentration (ccc), shown by the thick black line in Fig. 1 analogous to the cmc of surfactant solutions. As already emphasized, this line does not represent a true change of phase. Rather, it delimits a region over which behaviour changes markedly, from the pre-clustering region at very low concentrations to the cluster fluid region above the ccc. Within the pre-clustering region, clusters are extremely rare and the growth in the concentration of clusters is exponential, while in the cluster fluid this growth is approximately linear.

The width of this region becomes narrower as we move to the right in this diagram, as cluster size increases with increasing $A_a$.

At higher concentrations, we can expect to move from the cluster fluid to a cluster solid, although the precise nature of this transition remains unclear. In Fig. 1 this transition, denoted by the dashed line, is located approximately by treating clusters as effective hard spheres, and finding the concentration at which they would freeze. The effective hard sphere diameter, in this case, is determined by making use of the Barker-Henderson approximation[67] applied to the effective cluster-cluster interaction, which in turn is given by Eq. (5).

Increasing $A_a$ leads to larger, denser clusters. Although the theory sets the liquid-like density within clusters to be uniform, in reality this density will vary with distance from the cluster centre, where it will be greatest. Therefore, as $A_a$ increases we can expect the centres of clusters to become solid-like, or to freeze. Accordingly, we can also expect the diameter of the solid-like core of the cluster to grow with increasing $A_a$, although these expectations have yet to be confirmed. Moving further to the right in Fig. 1, we can expect the cluster size to eventually diverge, at which point bulk liquid-vapour separation is regained and the ccc narrows infinitesimally to become the binodal of a true first order phase transition.

Using this theory, analysis of the various contributions to the free energy for equilibrium cluster fluids shows that the equilibrium state coincides almost exactly with a minimum in the energy as cluster size is traded for cluster concentration[41]. Essentially, provided clusters are not too small, the entropy density displays a minimum that almost exactly coincides with the energy density maximum as cluster size is traded for cluster concentration at fixed overall density $\rho_b$. This allows a relatively simple approximation for the cluster size, $d_c$, to be developed based on considering the energy density only, which satisfies

$$\int_d^{d_c} dr r^3 \left(1 - \left(\frac{r}{d_c}\right)^2\right) \phi(r) g^{(2)}(r, \rho_l) = 0$$

where $g^{(2)}(r, \rho_l)$ is the radial distribution function for the bulk fluid at density $\rho_l$. Approximations to this rdf should produce a simple theory for equilibrium cluster size, which might prove useful in the design of materials based on SALR clustering.

Returning to the phase diagram in Figure 1, a completely novel feature is the existence of a first order cluster vapour-cluster liquid transition for a narrow range of SALR parameters. This transition occurs because the theory includes a term that accounts for the free energy of mixing of clusters and single particles. Under some conditions, therefore, it is favourable for the clusters and background vapour to de-mix.

Monte Carlo simulations performed in follow-up work show a transition from a cluster vapour to a condensed cluster phase does indeed exist[42]. Although the condensed cluster phase simulated re-

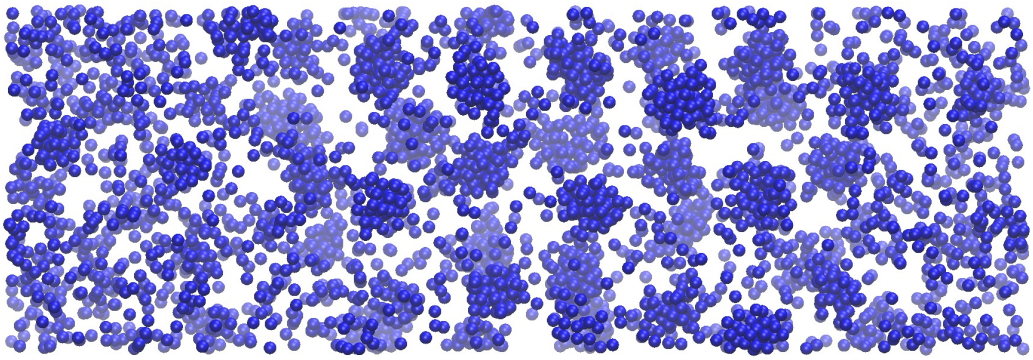

Figure 2. Snapshot from a Monte Carlo simulation of a SALR fluid phase separated into a cluster-rich phase, apparently crystalline with slab-like geometry due to periodic boundaries, bordered by a cluster-poor phase.

sembles a defective crystalline solid of clusters (see Figure 2), analysis of cluster-cluster interactions suggests a liquid phase might exist for a very narrow range of SALR parameters.

### B. Binary mixtures

Ferreiro-Rangel and Sweatman extended the theory discussed in section III for single-component SALR fluids to model binary mixtures of SALR and simple fluids, i.e. SALR + SA mixtures[44]. It is envisaged here that both components are dispersed within a solvent which, as before, is integrated-out and not treated explicitly. This model could be applied to treat, for example, solutions where the solute can exist in more than one charge state — a very common situation, particularly in biological systems.

Gibbs ensemble Monte Carlo simulations were used first to suggest a tentative phase diagram for this system, shown in Figure 3. These simulations did not incorporate Monte Carlo moves that attempt to transfer an entire cluster from one simulation box to the other, and so can only be used as a guide to more detailed studies. Nevertheless, they revealed four types of state; vapour, liquid, vapour with clusters and liquid with clusters. Clearly, the existence of each phase will be sensitive to SALR and SA model parameters, which were chosen in the simulations to generate a bulk vapour-liquid phase transition for the SA component by itself (i.e. the SA component is sub-critical) and a cluster fluid phase for the pure SALR component. The arrows in this diagram indicate the phase boundaries, which are only indicative, are expected to be sensitive to the cross-interaction between SALR and SA particles.

Results obtained from the theory support this general view of the phase behaviour of this binary mixture, although details of any first order cluster vapour - cluster liquid or solid phase transitions were not investigated. Perhaps the most interesting outcome of this work is that it was found that giant SALR clusters could exist even when they could not be formed by the SALR component by itself. In

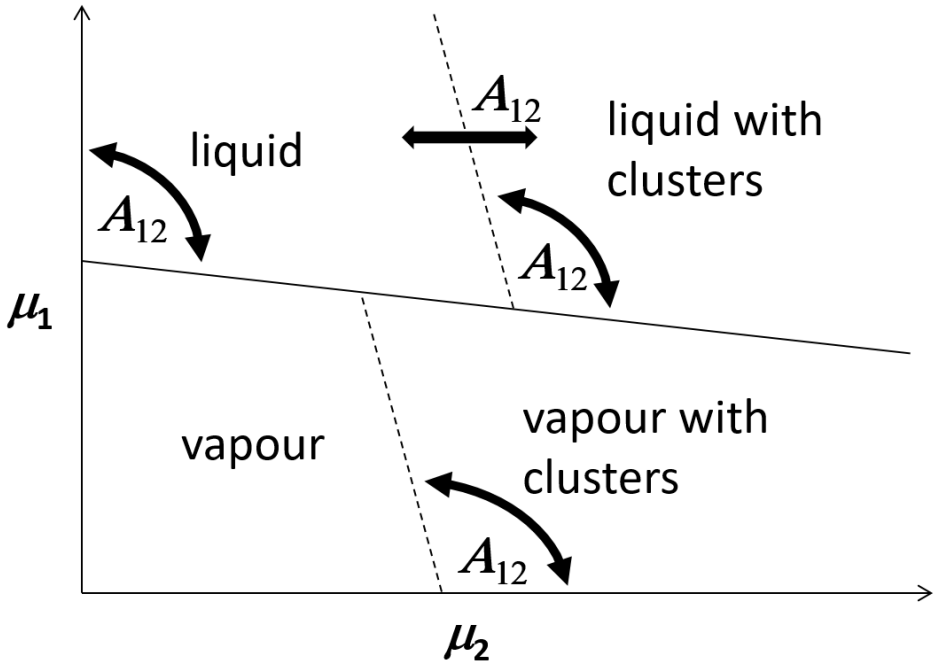


Figure 3. Tentative phase diagram for SALR + SA binary mixtures, indicated by Gibbs ensemble simulations, drawn in terms of the component chemical potentials, $\mu_1$ and $\mu_2$. Component 1 is the SA fluid while component 2 is the SALR fluid.

particular, the SA component could induce SALR clustering if the SA concentration was sufficiently high and the SA - SALR cross interaction was sufficiently attractive. In essence, it is possible for the SA component to generate an effective short-range attraction between SALR particles, sufficient to balance their long-range repulsion. This might have implications for many solutions, including biologically related ones such as aqueous amino acids, where the concentration of charged and neutral species can be adjusted by varying the pH, and their cross-interaction can be varied by adding salt.

## VI. GIANT SALR CLUSTER FISSIONING

Thus far we have focused on the equilibrium phase behaviour of giant SALR clusters and have seen that the disordered cluster fluid phase typically precedes the formation of more ordered aggregation structures at higher solute concentrations. We have also seen that the onset of clustering at low concentrations need not involve a first order phase transition. Like micellization, this type of change in fluid structure is sometimes called a pseudo-phase transition, characterised by a line (or more properly a narrow region) in the phase diagram at the critical concentration.

Of course, the formation of each giant SALR cluster is a dynamical process, and for larger clusters spontaneous cluster self-assembly from a homogeneous solution can occur via a nucleation event.

Classical nucleation arguments suggest the free energy barrier for this process increases, and therefore the rate of cluster nucleation decreases, exponentially with increasing equilibrium cluster size.

However, an alternative route to the formation of new clusters exists that avoids this high nucleation free energy barrier, and can therefore be much faster and dominant in many situations. Essentially, existing clusters can act as nucleation centres for the production of new clusters, i.e. heterogeneous nucleation is also possible. Specifically, it has been shown that under suitable conditions giant SALR clusters can split into two, or fission[68]. It goes without saying that this process is well known in nuclear physics, where the reverse process of fusion is also observed and understood. But in the field of soft and biological matter, this novel result has potentially profound consequences, especially for cell biophysics, synthetic life and the origin of life (see section IX).

Giant SALR cluster fissioning can occur readily in systems where the concentration of SALR particles slowly increases. Physically, this might correspond to slow evaporation of the solvent (as might occur in Darwins hypothetical warm little pond), or perhaps slow production of SALR particles via an in-situ chemical reaction (as might occur within a biological cell). As SALR particle concentration increases clusters can swell beyond their equilibrium size as they absorb more SALR particles. According to the phase diagram shown in Figure 1, to reach equilibrium the system should produce more clusters, yet the free energy barrier for homogeneous nucleation can be very high. Therefore the system can become trapped in a non-equilibrium state with a different number and size of clusters for the given overall system size and concentration. Recent work shows, using both the statistical thermodynamic theory described in section III and the simulation methods described in section IV, that in this situation the alternative pathway for cluster production. i.e. cluster fissioning, can then occur in preference to homogeneous nucleation.

Figure 4 illustrates what can happen. As a cluster grows beyond its equilibrium size, fluctuations in the cluster interface occur, eventually leading to a non-spherical prolate, or 'sausage-shaped' cluster. The cluster diameter typically remains close to the equilibrium cluster diameter, but the cluster can lengthen to around twice this size. Eventually, as the cluster grows further another fluctuation in the cluster interface occurs that forms a dumbbell or pinched shape. The two halves of the cluster can then separate to form two daughter clusters. Analysis of the single component theory in section III suggests the free energy barrier for this process is relatively low, and therefore it can dominate homogeneous nucleation. Indeed, it is the SALR analogue of a similar process known to occur for micelles[69] of aqueous surfactants.

However, Monte Carlo simulations mimicking Brownian Dynamics simulations show that this fissioning behaviour will only be observed if the concentration of particles is increased sufficiently slowly. For higher rates other processes can occur. This competition between cluster fissioning and other processes can be understood in terms of three timescales; i) the timescale for equilibration of

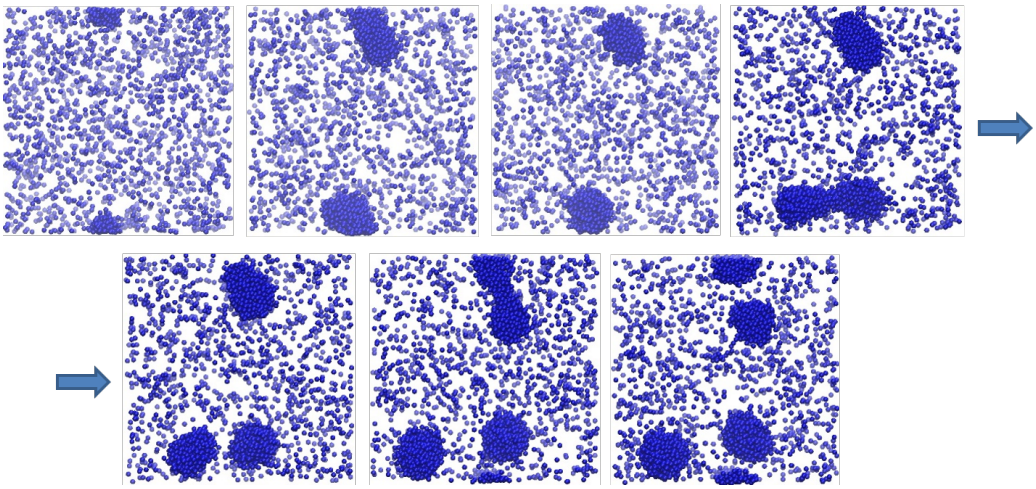

Figure 4. Snapshots from a 3D Monte Carlo simulation, mimicking Brownian Dynamics, illustrating growth and fissioning of a giant SALR cluster as concentration is slowly increased. The sequence starts at top-left and finishes bottom-right. Periodic boundaries apply, so the final frame has only four clusters.

particles within clusters, ii) the timescale for equilibration of clusters with the background solvent, and iii) the rate at which SALR particles accumulate in the system. Provided the accumulation rate of SALR particles is the slowest process then cluster fissioning can be expected to dominate. But if the rate of SALR particle accumulation is higher than the rate of equilibration of clusters with the background fluid then we can expect homogeneous nucleation to dominate. Finally, if the rate of particle accumulation is much slower than the rate of cluster equilibration with the background fluid, but much faster than the rate of internal cluster equilibration, then we can expect the formation of highly elongated clusters to dominate.

## VII. COMPLEX COACERVATES

When oppositely-charged polyions are mixed in solution, a variety of equilibrium phases can occur whose existence depends on the effective interactions between the solutes and their respective concentrations. In the simplest case, the polyions disperse, mimicking a vapour phase. Alternatively, bulk solvent-rich and solute-rich liquid phases might coexist. On the other hand, oppositely-charged polyions might aggregate to form a dispersion of small complexes with a reduced net charge, or, finally, it might happen that these complexes aggregate to form much larger clusters.

However, as for many other aggregating systems, this reasonably clear view of equilibrium behaviour can often be obscured by either slow dynamics or metastability. Understanding the equilibrium behaviour of these polyion mixtures, let alone disentangling it from the effects of kinetic frustration, has been a challenge for nearly a century. It is clear, though, that there appear to be many

parallels with aqueous surfactant solutions and SALR-like systems.

Confusion about the correct terminology for these systems has existed for some time. Certainly, the term 'complex coacervate' is mainly used to refer to equilibrium phases consisting of large, sometimes macroscopic, size-limited aggregates of polyions, but it has also been used to refer to dispersions of small complexes, as well as bulk phase separated systems. Again, the confusion likely stems from issues surrounding metastability and slow dynamics. Here, we use the term to refer only to the large, potentially macroscopic, size-limited equilibrium aggregates of complexes.

The overwhelmingly dominant view of this behaviour when large aggregates (typically on the scale of microns to millimeters) are formed is that it can be characterised as bulk liquid-liquid phase separation. While this view is certainly true for the case where bulk liquid phases develop, of course, it cannot be correct for complex coacervates as defined here, since the size of the liquid-like domains formed is limited. To be clear, it is simply not possible for a bulk liquid to form multiple separate domains at equilibrium, by definition.

We expect this situation arose because of the lack of an alternative mechanism for describing this behaviour when complex coacervation was initially investigated, more than 50 years ago. However, as the mechanism of cluster formation via a balance between long-range repulsion and short-range attraction, although not widely appreciated, is now firmly established, it is timely to revisit the problem of complex coacervation from this perspective. Indeed, the behaviour of polyion-surfactant mixtures has already been discussed in this light[70], but here we focus exclusively on oppositely-charged polyion mixtures that form complex coacervates.

At first sight, it might not be obvious that the SALR model is relevant to oppositely-charged polyion mixtures. After all, the SALR model is a one-component model where counter-ions are assumed to form a charge-neutralising dispersion and are not represented explicitly. However, it is well-known that in many cases macroscopic complex coacervates are observed to dissolve to form a dispersion of small molecular-scale complexes above a threshold temperature, and that this behaviour is reversible and repeatable under temperature cycling. Moreover, Kizilay et. al[70] emphasize the propensity of complex coacervates to form from small near-neutral complexes of oppositely-charged polyions. This provides a clue as to how complex coacervates might be modelled in terms of the SALR model.

Essentially, we expect that complex coacervates can be modelled as the aggregation of SALR particles, which themselves represent small near-neutral molecular-scale complexes formed by the association of oppositely-charged polyions. The effective SALR interaction arising between these small molecular complexes will depend sensitively on the free charge, stochiometry, structure and concentration of the individual polyions present in the mixture. Nevertheless, we can expect this view of these complex systems will be sufficient to reproduce the behaviour observed experimentally.

Taking this idea forward, we can then expect to observe SALR-like phase behaviour for complex coacervates (see Figures 1 and 3). That is, we can expect giant SALR clusters to be thermodynamically stable for a suitable balance between short-range attractions and long-range repulsions. However, at higher temperatures, or if long-range repulsions dominate, then we expect to see the giant clusters dissolve to form a dispersion of small complexes. Alternatively, if short-range attractions dominate, we expect to observe any clusters formed to gradually aggregate to form a separate bulk phase. Of course, much work remains to test this idea. If it is correct, it will mean that predictions of coacervate phase behaviour based on bulk liquid-liquid phase separation will only converge to correct results as cluster size diverges, as evident in Figure 1.

## VIII. MEMBRANELESS ORGANELLES

Biological cells are extremely complex microreactors. They cannot form spontaneously through homogeneous nucleation, but instead are created via fissioning. Whilst their chemical complexity, in terms of the reaction networks (or metabolic pathways) formed by their genetic material, proteins and lipids, has been explored extensively, much less work has been undertaken to understand their physical complexity[71].

The key issue that concerns us is the existence of many different kinds of membraneless organelle and signalling cluster within all cells. Membraneless organelles are liquid-like droplets formed by mixtures of specific proteins and RNA that carry out a wide range of important tasks within cells. For example, stress granules form when RNA is inhibited from carrying out its biological function of protein manufacture. Aggregates of RNA and protein are essentially stored in these membraneless "stress" droplets when the critical concentration of specific triggering proteins is exceeded. Cajal bodies, on the other hand, form inside the cell nucleus and, although their biological role is uncertain, it appears to at least be related to assembly of the ribozyme, the central protein manufacturing machinery of a cell.

For many years, the existence of these membraneless organelles was largely unexplained. Recently, however, as for complex coacervates, one physical theory for their existence has very quickly become totally dominant, namely bulk liquid-liquid phase separation[72]. This view stemmed from experiments showing these domains were liquid-like, and could coalesce, fission and distort as though liquid-like droplets. Since this apparent breakthrough, many attempts have been made to model their phase behaviour and properties in terms of bulk liquids.

However, just as for complex coacervates, this view might be wrong because it is not possible to form size-limited domains at equilibrium through the mechanism of bulk liquid-liquid phase separation. Neither can bulk liquid phase separation explain the apparent uniformity in size of these bodies,

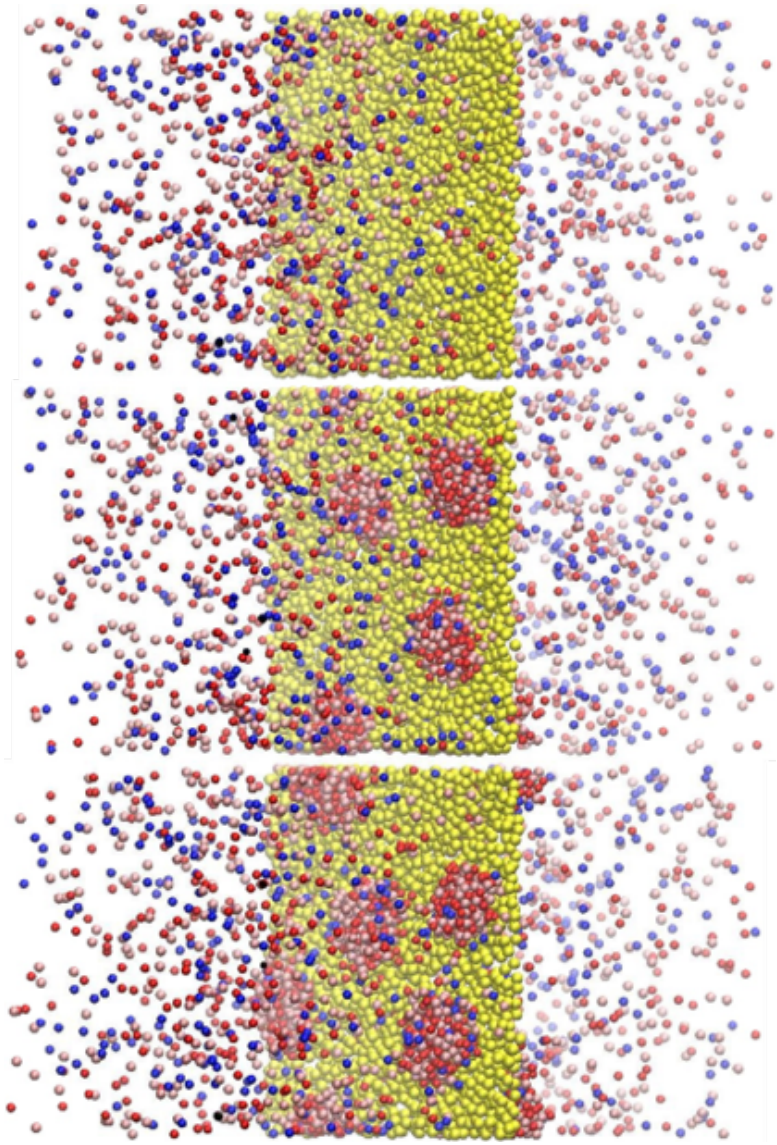

Figure 5. Snapshots from the end of Monte Carlo simulations modelling the WASP-membrane system with different protein-membrane interactions strengths (increasing in strength from top to bottom). Red and blue spheres represent active (SALR) and inactive (non-SALR) WASP proteins respectively, pink spheres are Nck (non-SALR) proteins, and yellow spheres are membrane particles (modelled as hard spheres). The simulation cell has been rotated to provide a good view.

or their specific lifetimes, or other physical behaviour such as crowding in a confined space without coalescing, a property of actin clusters. Quite possibly, however, their behaviour can be explained in terms of giant SALR clusters.

Recently, Sweatman and Insall used an SALR fluid to model the behaviour of one type of membraneless organelle, known as a WASP (Wiskott-Aldritch signalling protein) cluster, that plays a fundamental role in cell movement[73]. Within a cell, activated WASP and Nck proteins aggregate to form clusters on the interior of the cell membrane. Normally, the appearance of these clusters imme-

diately precedes the process of endocytosis, whereby the cell wall buckles inwards to form dimples, or 'puncta', which precede bulk movement of the cell wall. It is notable that these WASP-Nck clusters do not form in the interior of the cell, i.e. within the cytoplasm — they only appear to form discrete liquid-like aggregates with a specific size on the interior of cell membrane surfaces.

Figure 5 shows the results of Monte Carlo simulations modelling this behaviour using giant SALR clusters. This model is thought to be appropriate because it is known that, generally, proteins are charged in solution under biological conditions, and their mutual interactions and clustering behaviour have previously been modelled successfully in terms of the SALR interaction[74–76].

It was found that the appearance and surface density of giant SALR clusters formed by particles representing activated WASP and Nck proteins at a model membrane surface can be controlled by adjusting several system parameters, including the concentration of activated WASP protein in the bulk and its interaction strength with the membrane surface. Their appearance can also be controlled by the presence of a third protein at the membrane surface, mimicking the behaviour of clathrin proteins in cell membranes. In this latter case, the WASP-Nck clusters preferentially form around these surface-confined clathrin sites, mimicking experimentally observed behaviour.

Overall, the SALR model reproduces the basic physics of WASP-Nck membraneless organelles rather well. It provides a clear mechanism for the equilibrium formation of intra-cellular protein aggregates with specific sizes, as observed experimentally. The more commonly invoked mechanism of liquid-liquid phase separation cannot, by itself, explain how size-limited protein aggregates form with such fine control. As size-limited clusters are common in biology, this model might provide key insights into many important physical process inside cells.

## IX. LIFE VIA THE CHEMICAL EVOLUTION OF GIANT SALR CLUSTERS

The origin of life is one the most difficult and important problems in all science. There are two main approaches for tackling this problem, bottom-up and top-down[77], which also mirrors how we think about the origin of the Universe. A top-down "biological" approach begins with the most basic life-forms currently known and tries to draw conclusions about common characteristics to define life itself. It then considers how the postulated common ancestor to all life, known as LUCA (the Last Universal Common Ancestor), might have evolved from a slightly simpler organism, *ad infinitum*. This approach, often employing synthetic biology[78], typically leads to suggestions that life began with the first cell composed of genetic material surrounded by a lipid membrane[79,80]. But this is already a very complex organism, and this line of reasoning therefore leads to a paradox: how can a living organism of this complexity assemble spontaneously? As this scenario appears unlikely, the alternative should be considered; life began with an organism even simpler than the most basic cell

imaginable. The problem is that it is difficult to conceive of a functioning organism without both these apparently essential properties; genetic material and a lipid membrane. It appears new physics would help to resolve this paradox[81–84].

The alternative, bottom-up "geochemical" approach considers the chemical pathways that might have led to life on an early Earth. Many have been suggested, with the main theories known as an "x-world" hypothesis. For example, the RNA-world hypothesis proposes that life began with the spontaneous self-assembly of RNA[85,86], while the membrane-world hypothesis proposes that life began with the formation of micelles or lipid vesicles[87]. But each hypothesis has its problems to overcome, which currently seem insurmountable. For example, the RNA-world lacks a mechanism for evolution at the level of the organism since there is no organism, just RNA (or RNA pairs) dispersed in solution. This hypothesis, therefore, does not address the problem of how a functioning organism can evolve, which is unsatisfactory for a theory for the origin of life.

The membrane-world hypothesis, on the other hand, lacks a mechanism for evolving an autocatalytic network. An autocatalytic set of reactions is one where all catalysts required by the reaction set are produced by the reaction set[88]. Their behaviour is very stable chemically, i.e. they can tolerate strong chemical perturbations so that the products are generated reliably. Biology uses autocatalytic reaction sets with genetic storage of information to ensure offspring are reproduced with a tolerably-low level of genetic errors and are therefore viable. Since membrane lipids do not store chemical information in a suitable form, i.e. genetically, they cannot chemically evolve. We are back to the same paradox as before.

The same problems arise for each "world" hypothesis in isolation. Fundamentally, the problem is that no single class of molecule currently known can both form an autocatalytic set (so that chemical information is accurately reproduced) and spontaneously self-assemble to form a self-contained organism on which evolution (at the level of the organism) can act.

A more holistic hypothesis is that the ingredients apparently needed for life, e.g. RNA, proteins and perhaps lipid membranes, somehow co-assembled and co-evolved together from the "primordial soup", although the physicochemical pathway remains obscure[89,90]. The key advantage of this paradigm is simultaneous evolution of the genetic chemistry together with a physical mechanism for self-assembly, so that evolution at the level of the organism occurs. To date, the most promising research along these lines is perhaps by Koga et al., who have demonstrated that short cationic peptides and phosphorylated mono-nucleotides can co-assemble to form stable liquid-like droplets[91]. This is a very important step, as it indicates how the building-blocks of life (nucleotides form RNA and DNA, while short peptides can be viewed as primitive proteins) can self-assemble to form stable clusters or aggregates, which might be regarded as very primitive precursors of an organism.

Koga et al.'s droplets form because the positively charged peptides and negatively charged phos-

phorylated nucleotides strongly attract each other. In other words, their clusters are types of complex coacervate and can therefore be considered as a kind of giant SALR cluster. Consequently, it follows from section VI that they also can be expected to also undergo fission events similar to cell division if the solute concentration is slowly increased.

Essentially, both the required chemistry and physics for biological reproduction might be displayed by this type of system. That is, these specific complex coacervates include the essential chemical building-blocks of life (i.e. DNA/RNA and proteins), and they are assembled into clusters likely to display growth and fissioning under suitable conditions. However, Koga et al. did not report on specific observations of either droplet fissioning or mononucleotide chemistry, because this was not their aim.

Unfortunately, their work remains unique to date — there have not been any further studies of these specific systems, or simpler ones that, for example, involve amino acids rather than short peptides. Therefore, much work remains to show that this type of system can evolve chemically and is therefore a good candidate for the origin of life. Nevertheless, their work does provide important insights.

The possibility that complex coacervates might be important in the origin of life was first suggested by Oparin in the 1920s[92]. The fissioning behaviour of specific coacervates reminded him of biological cell reproduction. But the coacervates he studied typically involved large unreactive polyions, such as gum arabic, which are unlikely to be directly relevant to the origin of life. It is for this reason that the work by Koga et al. is particularly interesting, as it uses biologically relevant molecules. Another reason is that they showed that other organic molecules preferentially partition to the organic microphase. Therefore, each coacervate droplet has the potential to behave as a microscopic reactor in which the reactants are concentrated.

Another potential clue to the origin of life was provided by the work of Sydney Fox and his collaborators from the 1960s onwards[93]. They found that amino acids heated to fairly high temperature underwent condensation reactions to form a peptide gum, which after being cooled, powdered and dispersed in water formed what they called “proteinoid microspheres”. These microscopic particles could often be observed to swell and fission under a microscope. This fissioning behaviour led Fox to develop his protein-world theory for the origin of life. However, his work was overtaken by developments in genetics, and consequently the search for the origin of life moved decisively towards exploring the DNA-world and then RNA-world hypotheses.

Both Oparin and Fox saw the parallels between the their specific systems and biological reproduction, i.e. the ability to spontaneously fission. We suggest their systems are likely to be specific cases of the more general SALR model fluid. Therefore, any system of the SALR type that also involves molecules able to store and evolve chemical information might be a good candidate system for the origin of life or synthetic life.

Let us suppose that such a system is discovered. Perhaps, following Koga et al.[91], it might consist of a simple mixture of cationic amino acids and phosphorylated mononucleotides. And let us suppose that this system displays fissioning behaviour, as expected for giant SALR clusters, under suitable conditions (perhaps through solvent evaporation cycles, for example). If the clusters store chemical information such that, together with a cluster "fitness" selection process, chemical evolution occurs, the obvious question would then be "Is this life"?

An attempt to answer this question was provided in earlier work[68]. It concluded that once giant SALR clusters spontaneously evolve an autocatalytic reaction network and becomes so complex that homogeneous nucleation is practically impossible, they could be considered to be alive.

## X. SUMMARY

Our interest in the chemical physics of giant SALR clusters was initially stimulated by observations of giant, apparently equilibrium, clusters in aqueous amino acid solutions[94–96]. This matter remains unresolved, in that it is still not clear whether the clusters observed contain any amino acid, or are instead clusters of some unspecified impurity, and neither is a mechanism, presuming they are composed solely of amino acid, for their formation understood. But throughout this research we have focused on the possibility that they might be explained in terms of giant SALR clusters.

This research led to the development of a novel theory of aggregation for SALR fluids that uniquely (i) is based on microscopic parameters appearing in the Hamiltonian, and (ii) includes a term for the free energy of mixing of clusters and individual particles in the background 'vapour'. This approach successfully describes the general features of micelle-like behaviour, but also predicted a new first order phase transition, from a cluster vapour to a condensed cluster phase, which was subsequently observed in later Monte Carlo simulations. The alternative approach, based on liquid state integral equation theory, has not yet been able to describe this behaviour, and so appears to require further fundamental developments in the specification of suitable bridge functions.

Efforts to extend this theory to more complex systems, for example those formed by mixtures or at higher concentration where ordered structures appear, have begun but are in their infancy, and much work remains. Despite its limited successes, this approach clearly lacks 'resolution' and versatility, being based on a simple mono-disperse view of clusters with uniform internal density, and therefore more accurate treatments are desirable.

Nevertheless, this theory has extended our understanding of the basic physics of equilibrium clustering in solution, and appears to have direct relevance to many soft matter systems where this kind of clustering takes place. Applications to biologically-related systems, such as membraneless organelles and complex coacervates, are especially pertinent considering that the current paradigm for their ex-

istence, based on bulk liquid-liquid phase separation, appears to be inadequate in several respects.

Indeed, given (i) the apparent existence of giant amino acid clusters in aqueous solutions, (ii) the known clustering behaviour of many protein solutions, (iii) the discovery of giant SALR cluster fissioning behaviour, (iv) its apparent success in describing the behaviour of a membraneless organelle (specifically WASP-Nck clusters), and (v) its potential application to complex coacervates, which have been implicated in the origin of life since Oparin's suggestions in the 1920s, the phenomenon of giant SALR clustering might also have an important role to play in the origin of life and studies of synthetic life. It might provide a resolution to apparent paradoxes concerned with the transition of non-living to living matter.

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